RESPONSE TO OFFICE ACTION

A. Status of the Claims

The Action notes that claim 11 has been withdrawn from consideration. In response, Applicants note that the claim has been canceled herein. Applicants reserve the right to prosecute non-elected subject matter in more or more continuing applications. Claims 1 and 10 have been amended. Claims 1-10 and 12-13 are pending and presented for reconsideration.

B. Rejections Under 35 U.S.C. § 101

The Action rejects claims 1 and 10 as reading on products of nature. For example, it is stated that claim 1 reads on sequences found in their native state in a genome and claim 10 reads on non-transgenic seeds. In response Applicants note that claim 1 has been corrected by specifying that the promoter in claim 1 is "isolated." It is noted that the claimed nucleic acids need be isolated only with respect to their natural and unaltered condition in a plant genome, but need not be separate from any other given sequence and can be combined with any other sequences and introduced into plant or other genomes, as explained in the specification.

With regard to claim 10 it is noted that the claim has been amended to specify that the claimed seed is transgenic.

The amendments do not narrow the claims as the amended limitations were inherent in the claims as filed. In view of the foregoing, the rejections are moot and removal thereof is thus respectfully requested.

C. Rejections Under 35 U.S.C. § 112, First Paragraph - Enablement

The Action rejects claims 1-10 and 12-13 as lacking enablement. In particular, the Examiner acknowledges that enablement is present for the full length promoter sequence of SEQ ID NO:4, but asserts that enablement is lacking for promoter sequences comprising a fragment of SEQ ID NO:4 or sequences with 70% identity to the full length sequence or fragments thereof having promoter activity. In support of the rejection it is asserted that Kim *et al.* (1994; *Plant Molecular Biology*, 24:105-177) show that "mutation of a single nucleotide significantly altered the strength of expression, while deletions in other regions of the promoter completely eliminated function." Action at p. 4, citing page 108, first full para. of Kim *et al.* The Action also cited the Dolferus *et al.* article (1994; *Plant Physiology* 105:1075-1087) for the assertion that the authors found in a deletion analysis of the *Arabidopsis Adh* promoter that "deletion of different elements of the promoter affected promoter function conditional to the stress that was applied to the given promoter fragment." Action at p. 5, citing p. 1080, last full paragraph and page 1082 first full paragraph of Dolferus *et al.*)."

With respect to the Kim *et al.* reference, Applicants initially note that the promoter discussed in this paper is the *nos* promoter from the bacteria *Agrobacterium tumefaciens*. This is not a promoter from a plant and thus any negative inferences attempted with respect to the promoter of SEQ ID NO:4, which was isolated from the plant species *Arabidopsis thaliana*, are not scientifically well-founded. As explained in the first and second sentences of the Introduction section of Kim *et al.* with regard to the T-DNA that includes the *nos* promoter at issue, "[b]ecause several genes are clustered within a short segment of the T-DNA, the regulatory regions of each gene are relatively small compared to those of plant genes" even though typical eukaryotic regulatory sequences are present. It is thus not surprising that some

essential promoter elements would be found in such a compact regulatory element. Despite this, the authors demonstrate that numerous mutations can be made even in the critical promoter region focused on between position -131 and -112. For example, it is explained that low level promoter activity was still obtained "even when the entire hexamer sequence was removed (oligomer 125-112)." Kim *et al.* at p. 109, 1st col, bottom ¶. Table 2 further shows numerous different point mutations that were created, all but two of which still retained promoter function. It must further be noted that Kim *et al.* focused on the regions of the promoter most critical to function and sought out those deletion fragments that had no activity. Kim *et al.* therefore shows that as of at least the ***1993*** publication date those of skill in the are could make substantial modifications to the most critical regions of even a compact promoter of bacterial origin while still retaining promoter function. This reference therefore more than adequately demonstrates that as of the filing date is was routine for those of skill in the art to create promoter deletion and mutation fragments retaining promoter activity.

The Dolferus reference even more convincingly demonstrates enablement, as it related to a promoter of plant origin. The authors show that deletion of a section from -964 to -510 of the 1kb (-964 to +53) promoter region of the Adh gene "results in ***increased expression*** under uninduced and all stress conditions, suggesting that this region contains a repressor binding site." (emphasis added) See Abstract of Dolferus *et al.* Therefore, this alone shows that well over 30% of the promoter could be deleted altogether while obtaining an even more effective promoter element. With respect to the assertion that some deletion fragments "affected promoter function conditional to the stress that was applied to the given promoter fragment" this is irrelevant to the claims because the claims do not require inducible or other expression profiles and promoter activity was retained. The reference therefore again established enablement by showing that as

of at least *1994* it was routine in the art to mutate and delete over 30% of a promoter while still retaining promoter function.

Applicants further note that the teaching in the specification and numerous other art references demonstrate that it would be routine in the art as of the filing date to make and use claimed fragments and 70% identical sequences having promoter activity, for example, as described in the specification at page 9, line 10 and following, and page 10, line 6 and following. The specification, for example, explains that fragments of a starting promoter sequence such as SEQ ID NO:4 can routinely be generated by using restriction digests with various endonucleases, or any other desired method. Fragments generated can routinely be screened for activity by expression assays, for example, using the expression construct prepared according to working Example 4 of the specification and the expression assays, the GUS reporter gene according to working Example 5, and pMON65422 of working Example 6. Those fragments having the desired activity can routinely be identified because only a limited number of fragments are required that overlap the entire length of SEQ ID NO:4, given that it is less than 1 kb in length.

The specification also teaches that variants of SEQ ID NO:4 having 70% or more identity to this sequence can be generated by well known techniques, for example, by insertion, deletion, or replacement of template sequences in a PCR-based DNA modification approach (*e.g.* p. 9, lines 10-28). Chimeric and variant promoters can also be generated (see for example, U.S. Patent Nos. 4,990,607; 5,110,732; and 5,097,025). This can be guided, as explained in the specification (*e.g.* page 6, lines 5-17), by use of well known methods including mapping of desired promoter elements, such as by DNA binding protein analysis using DNase I footprinting, methylation interference, electrophoresis mobility-shift assays, in vivo genomic footprinting by

ligation-mediated PCR, and other conventional assays; or by DNA sequence similarity analysis with known cis-element motifs by conventional DNA sequence comparison methods.

Any doubts that such variants could be recovered having promoter activity are dispelled by the numerous references in the art demonstrating that substantial deletions and changes can be made to a promoter while still retaining promoter activity. In addition to the references cited in the Action, Applicants respectfully direct attention to **Exhibits A-C**, which describe the alteration and modification of promoter sequences. Welsch *et al.*, for example, demonstrated that approximately 89% of a starting promoter sequence could be deleted while still retaining promoter activity and at least 24% could be deleted without appreciably altering activity. **Exhibit A** at p. 526, FIG. 1 and 2nd col. Piechulla *et al.* showed that approximately 96%, 81%, 54% and 53% of four promoter sequences analyzed could be deleted while still retaining promoter activity, even if decreased or somewhat altered. **Exhibit B**; p. 659, Fig. 5. Cho and Cosgrove showed that at least 69% and 73% of two promoters analyzed could be deleted without significantly affecting promoter activity, and further that numerous substitution mutations could be made in a fragment of the former promoter while still retaining full activity and in some cases increasing activity. **Exhibit C**, p. 3244, 2nd col., FIGs. 8-10, p. 3245, 2nd col. The reference of Dolferus *et al.* cited in the Action (*e.g.* at page 1079, last paragraph, to page 1082, first paragraph; figure 4; and table I) also describes several Adh promoter deletion constructs that have no significant effect on expression levels. In particular, 5' deletion up to position -575 (*i.e.* approximately 40% of the sequence) had no significant effect on expression, and further deletions to -309 (*i.e.* deleting approximately 70% of the original sequence) retained detectable promoter activity.

It would therefore be a straightforward matter for one of skill in the art to generate subfragments of SEQ ID NO:4 and 70% identical sequences that retain promoter activity, especially given the detailed teachings in the specification and knowledge in the art. While this would require some routine screening, "[e]nablement is not precluded by the necessity for some experimentation such as routine screening." *In re Wands*, 858 F.2d at 737. Where the specification "provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed" this does not constitute undue experimentation. *Id.* (quoting *Ex parte Jackson*, 217 USPQ2d 804, 807 (Bd. App. 1982)). This is underscored by the fact that those of skill in the art knew how to make such routine changes as of filing date. Therefore, given the detailed teaching in the specification, advanced state of the art and scope of claimed subject matter, compliance with the enablement requirement has been fully demonstrated.

Removal of the rejection under 35 U.S.C. §112, first paragraph, is thus respectfully requested.

D. Rejections Under 35 U.S.C. § 112, First Paragraph – Written Description

The Action rejects claims 1-10 and 12-13 for failing to comply with the written description requirement of 35 U.S.C. § 112, first paragraph. Specifically, the Action asserts that written description has been provided for SEQ ID NO:4, but not for fragments or 70% identical sequences thereof. Applicants respectfully traverse as set forth below.

Applicants were in possession of the full scope of claimed subject matter as demonstrated in the specification and thus the claims do not lack written description. First, all fragments of SEQ ID NO:4 have literal written support in the sequence listing. That is, the claimed fragments

of SEQ ID NO:4 require no more sequence information than is present in the sequence listing. Written description cannot be lacking for what is literally described. As explained in the specification, fragments of SEQ ID NO:4 can be generated by methods well known in the art, for instance by restriction digests, physical shearing or any other method for breaking the DNA backbone. Promoter activity can be routinely confirmed by expression assays, for example, using the expression construct prepared according to working Example 4 of the specification and the assays according to working Example 5. As shown above, this was routine in the art, which must be taken into account in the written description analysis as discussed further below.

With respect to sequences with 70% identity, these are also fully described in the specification. First, the genus of claimed sequences is limited to those having 70% identity to SEQ ID NO:4, and thus there is a finite scope of claimed subject matter which is supported by the description in the sequence listing. In addition, the specification fully describes the creation of variants of SEQ ID NO:4. For example, the specification explains that creation of derivatives of a promoter can guided by mapping of any desired promoter elements, such as by DNA binding protein analysis using DNase I footprinting, methylation interference, electrophoresis mobility-shift assays, *in vivo* genomic footprinting by ligation-mediated PCR, and other conventional assays; or by DNA sequence similarity analysis with known cis-element motifs by conventional DNA sequence comparison methods (*e.g.* specification, p. 6, lines 5-17). The specification also describes, for example, the use of PCR to modify selected nucleotides or regions of sequences. As explained, the sequences can be modified by insertion, deletion, or replacement. The specification also describes known methods for construction of chimeric and variant promoters (see for example, U.S. Patent Nos. 4,990,607; 5,110,732; and 5,097,025).

It must further be noted that written description is reviewed from the perspective of one of skill in the art at the time the application is filed. *Wang Labs., Inc. v. Toshiba Corp.*, 993 F.2d 858, 863 (Fed. Cir. 1993). As of the filing date, it was well known that substantial alterations can be made to a promoter sequence while retaining promoter activity. In this regard, the specification incorporates numerous publications describing modification of promoters (*e.g.* specification page 10, line 9. In addition, Applicants direct attention to **Exhibits A-C**, which describe the alteration and modification of promoter sequences. For example, Welsch *et al.* (**Exhibit A**; "Structural and functional characterization of the phytoene synthase promoter from *Arabidopsis thaliana*"; *Planta* 216: 523–534 (2003)) describe the creation of multiple deletion fragments of an *Arabidopsis thaliana* phytoene synthase (*psy*) gene promoter. Starting with a full length sequence comprising 1746 nucleotides of the 5' region, truncations were created and tested for expression comprising only 1314, 910, 809, 300 and 196 nucleotides of the 5' upstream region. **Exhibit A** at p. 526, FIG. 1. It was shown that the deletion comprising 1314 nucleotides was "almost indistinguishable" in the pattern of expression. *Id.* at p. 526, 2nd col. It was also shown that, while truncation to 196 nucleotides abolished the responsiveness observed in the full length promoter to some types of light, responsiveness (*e.g.*, promoter activity) was still observed to several types of light. *Id.* Therefore, the authors showed that at least 432/1746 (*e.g.*, 24%) nucleotides could be deleted from the full length promoter with essentially no change in expression and at least 1550/1746 (*e.g.*, 89%) nucleotides could be deleted while still retaining promoter activity.

Similarly, Piechulla *et al.* (**Exhibit B**; "Identification of tomato Lhc promoter regions necessary for circadian expression" *Plant Molecular Biology* 38: 655–662, 1998) describe the deletion analysis of promoters from the cab 1A, cab 1B, cab 8 and cab 11 genes from the tomato

light harvesting complex of genes to determine which deletions would affect circadian expression. Deletion constructs were tested comprising from between 1091 and 43, from between 793 and 152, from between 322 and 148 and from between 251 to 119 nucleotides from these promoters, respectively. **Exhibit B**, p. 659, Fig. 5. As explained by the authors, the "results show that the short 5'-upstream regions are sufficient for a basal mRNA accumulation" and further indicate that upstream sequences are responsible for circadian rhythm. The results therefore indicate that at least 1048/1091 (*e.g.*, 96%), 641/793 (*e.g.*, 81%), 174/322 (*e.g.*, 54%) and 132/251 (*e.g.*, 53%) of the nucleotides of these promoters could be deleted while still retaining promoter activity.

The foregoing is also illustrated by other studies, such as that of Cho and Cosgrove (**Exhibit C**, *Plant Cell*, 14, 3237–3253, 2002). These authors showed that more than 990 base pairs of an approximately 1428 bp plant promoter sequence designated AtEXP7 could be deleted without significantly effecting promoter activity and even larger deletions could be made while maintaining a reduced promoter activity. See **Exhibit C**, p. 3244, 2nd col. and FIG. 8. It was also shown that a deletion of approximately 775 bp could be made from a 1058 bp plant promoter designated AtEXP18 without significantly reducing promoter activity. See *Id.* at FIG. 10. Finally, the authors further showed that numerous substitution mutations could be made in a fragment of AtEXP7, while retaining full promoter activity and in some cases increasing activity. See. *Id.* at FIG. 9 and p. 3245, 2nd col. These studies therefore show that fragments of full length promoter sequences can routinely be made that retain promoter activity.

The foregoing therefore demonstrates that Applicants were in possession of the invention. Applicants need not show how or why the claimed promoter sequences function, for example, by specifying which functional elements cause promoter activity. What is relevant under 35 U.S.C.

§ 112, first paragraph, is that Applicants were in possession of the ***claimed invention***. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64 (Fed. Cir. 1991). The claims are not directed to particular functional elements, but rather to nucleic acids having promoter function. Written description for the claimed invention, is therefore present based on the description of SEQ ID NO:4 and accompanying detailed descriptions and teachings in the specification when properly viewed in the context of the knowledge in the prior art, as required under Federal Circuit precedent. Withdrawal of the rejection under 35 U.S.C. § 112, first paragraph is thus respectfully requested.

E. Rejection Under 35 U.S.C. § 112, Second Paragraph –Indefiniteness

The Action rejects claims 1-10 and 12-13 under 35 U.S.C. § 112, Second Paragraph, as being indefinite, in that the term "fragment" in claim 1 is not clearly defined. Applicants respectfully traverse as the term used has a plain meaning well known in the art. For example, the online version of the Merriam Webster™ Dictionary (http://www.m-w.com/) gives the meaning of the term "fragment" as "a part broken off, detached, or incomplete." In addition, the assertion that the length is indefinite is without basis because the claim requires the fragment to have promoter activity, and thus the term is not without length limitation. In sum, one of skill in the art in possession of the specification would clearly understand the metes and bounds of the claims and thus §112, second paragraph has been fully complied with. Withdrawal of the rejection is thus respectfully requested.

F. Rejection Under 35 U.S.C. § 102

(1) The Action rejects claim 1 as anticipated by Johnson-Hopson, *et al.* (GenBank Accession AC005106). In particular it is asserted that Johnson-Hopson, *et al.* teaches a polynucleotide sequence with between 90 and 99% identity to SEQ ID NO:4, *e.g.*, 92.7% identity, and thus anticipates the claims as the ability of the sequence to function as a promoter would have been an inherent property. In response, Applicants note that claim 1 is directed to an isolated promoter sequence operably linked to a transcribable polynucleotide molecule. This element is not taught or suggested by the cited reference, which instead is described as a "genomic sequence from *Arabidopsis thalina* BAC T25N20 from chromosome I, complete sequence". This 84,181 nucleotide sequence is without annotation of promoters, does not appear to teach any function for the allegedly transcribable polynucleotide downstream of this sequence, or indicate that a resulting polypeptide is even expressed. Thus an isolated promoter sequence as claimed is not taught or suggested by the cited reference. Because there is no teaching of all elements of the claims or a suggestion to arrive at the claimed nucleic acids, removal of the rejection is respectfully requested.

(2) The Action next rejects claims 1-10 and 12 under 35 U.S.C. §102(b) as anticipated by McElroy *et al.* (U.S. Patent 6,207,879). In particular it is asserted that the RS81 promoter taught by McElroy *et al.* would inherently comprise a fragment of at least one base pair of SEQ ID NO:4 or any sequence with at least 70% identity thereto and thus anticipates the claims. Further, the Action alleges that "the term 'fragment' may reasonably be interpreted to include a single base pair".

In response, Applicants note that a BLAST sequence alignment of SEQ ID NO:4 with the RS81 promoter (SEQ ID NO:1) of U.S. Patent 6,207,879 found no significant similarity between

the two sequences using default parameters (**Exhibit D**). Further, the claimed invention is directed to a promoter. As explained for instance at page 10, lines 15-17 of the specification, a promoter includes a length of polynucleotide sequence that is capable of regulating an operably linked transcribable sequence. A single base pair fragment as cited in the specification would ***not*** comprise promoter activity and no basis for such conclusions has been presented. This is illustrated by the references attached as Exhibits A-C, which show that while substantial modifications can be made to a promoter element, some promoter sequence is needed for activity and in no instance is this a single nucleotide. If a single nucleotide could serve as a promoter then every occurrence of that nucleotide in the genome would constitute a promoter. It goes without saying that plants do not contain the millions of promoters that would result from this in a typical plant genome that exceeds hundreds of millions of base pairs. Removal of the rejection is thus respectfully requested.

(3) Finally, the Action rejects claims 1-3, 5-7, 10, 12 and 13 under 35 U.S.C. § 102(b) as anticipated by Debonte *et al.* (U.S. 5,850,026). For example it is asserted that a promoter described by Debonte *et al.* "would inherently comprise a fragment of at least one base pair of SEQ ID NO:4 or any sequence with at least 70% identity thereto." It is also alleged that the reference teaches methods of producing oil and meal.

In response Applicants note as stated above that the claimed invention is directed to promoter sequences and a promoter must be capable of regulating expression of an operably linked transcribable sequence. The teachings of the specification and the knowledge of one of skill in the art as shown in **Exhibits A-C** demonstrate that a one base pair fragment is not properly considered to be a promoter, let alone a sequence according to claim 1. Indeed no defined promoter nucleotide sequence appears to be specifically disclosed in Debonte, or

referred to in the rejection. Instead, the Debonte sequence listing appears to contain only coding nucleotide sequences or polypeptide sequences.

Further, the use of the term "fragment" in Debonte is distinguishable from the present application. In Debonte, "regulatory fragment" refers not to a fragment of a promoter, but rather to a fragment ***of a construct*** that also comprises, for instance, a coding sequence fragment. No fragments of a promoter are shown to be present anywhere in the specification of Debonte. The cited reference therefore simply does not relate to, or teach, any fragment of a promoter, let alone a sequence according to claim 1. Thus the reference does not anticipate the claims and withdrawal of the rejection is respectfully requested.

G. Conclusion

The Examiner is invited to contact the undersigned attorney at (512) 536-3085 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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